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Antidiabetic effect of aqueous extract of *Basella alba* leaves and metformin in alloxan-induced diabetic albino rats

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The present study was carried out to evaluate the anti-diabetic effect of aqueous extract of *Basella alba* leaves in alloxan-induced diabetic albino rats. Thirty (30) male albino rats weighing 100 to 150 g were used for this work. The rats were randomly distributed into five groups containing six (6) rats per group. Group I rats served as the healthy control group and were neither induced with diabetes nor given any treatment. Group II rats served as the diabetic control. They were induced with diabetes but not given any treatment throughout the experiment. Rats in group III and IV were induced with diabetes and administered *B. alba* leaf extract at different doses, 100 and 200 mg/kg, respectively. Group V rats were also induced with diabetes and treated with Metformin at a dose of 100 mg/kg body weight. The various treatments were administered orally for a period of three weeks. The mean fasting blood glucose (FBG) of the rats was determined weekly using a glucometer. The rats treated with *B. alba* at doses of 100 and 200 mg/kg had their mean FBG levels significantly lower than the diabetic control group ($p<0.05$). Similarly, there was significant difference between rats in the group treated with Metformin and the diabetic control group ($p<0.05$). The mean FBG levels of rats treated with 200 mg/kg of *B. alba* leaf extract was not statistically different from that recorded in rats treated with Metformin. The results show that *B. alba* leaf extract has antidiabetic effect in alloxan-induced diabetic rats, varying with the quantity ingested. Hence, consumption of *B. alba* leaf as a vegetable should be encouraged for the treatment of diabetes mellitus.

Key words: *Basella alba*, diabetes mellitus, fasting blood glucose, metformin, hypoglycemia.

INTRODUCTION

Diabetes mellitus is a major worldwide health problem involving endocrine pancreas. It is implicated in oxidative stress which induces insulin resistance in the peripheral tissue and impairs insulin secretion from pancreatic β-cells (Ceriellos and Mortz, 2004; Wang et al., 2006). It is a major cause of adult blindness, kidney failure, neuropathy, heart attack and strokes. It is also characterized by excessive disturbance of carbohydrates, proteins and lipid metabolism, thickening of capillary basement membrane throughout the body leading to microangiopathy, macroangiopathy and long term complications which affect eyes, kidneys, nervous system and circulatory

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system (Dhasarathan et al., 2011). Due to increasing obesity and altered dietary habits in both western and developing countries, the prevalence of type 2 diabetes is growing at an exponential rate (Zimmet and Lefebvre, 1996). It has become a major menace in the last 10 years. In 2004, according to the World Health Organization (WHO) more than 150 million people worldwide suffer from diabetes. The WHO has predicted that the major burden will occur in developing countries. Life expectancy may be halved by diabetes mellitus especially in developing countries where its prevalence is increasing and adequate treatment is often unavailable (Dhasarathan et al., 2011).

Plants have been the major source of drugs for the treatment of diabetes mellitus in some countries of the world like India and China (Dhasarathan et al., 2011). The importance of antidiabetic plants in the development of economic and effective treatment for diabetes currently estimated to affect over 30 million people worldwide has been recognized by the World Health Organization (WHO Technical Report Series, 1985). Most of the anti-diabetic plants have been found to contain substance like glycolsides, alkaloids, terpenoids, flavonoids and so on (Loew and Kaszkin, 2002). The ethnobotanical information reports stated that about 800 plants may possess anti-diabetic potential (Aquilara et al., 1998). Among the plants reported, *Basella alba* may be included in that it contains most of the substances possessed by the already discovered anti-diabetic plants but not yet scientifically proven to possess the potential. *B. alba* (family *Basellaceae*), is a fast growing vegetable, native to tropical Asia, probably originating from india and Indonesia and extremely heat tolerant areas (Grubben and Denton, 2004). It is grown throughout the tropics as a perennial plant and in warmer temperate regions as an annual crop. Its thick semi-succulent heart-shaped leaves have a mild flavour and mucilaginous texture. It is commonly known as Malabar, Ceylon, East-Indian, Surinam and Chinese Spinach (Facciola, 1990). It is known to be high in vitamin A, vitamin C, vitamin B9 (Folic acid), Calcium, Magnesium and several vital anti-oxidants. It is low in calories by volume and high in protein per calories (Duke and Ayensu, 1985).

The flowers are used as an antidote for poisons (Duke and Ayensu, 1985). It is also a safe aperient for pregnant women and its decoction has been used to alleviate labour (Duke and Ayensu, 1985). The aqueous extract of *B. alba* leaves has been reported to reduce anaemia and maintain good health (Bamidele et al., 2010). *B. alba* traditionally claimed to increase libido (Kuete and Efferth, 2010). A recent literature survey revealed that the plant extract possess analgesic, (Anandarajagopal et al., 2011) anti inflammatory activities (Chaitanya, 2012), microbial activities (Oyewole and Kalejaiye, 2012), gastro protective action (Kumar et al., 2012) and CNS depressant activity (Anandarajagopal et al., 2011). Species of the plant called *Basella rubra* has been scientifically investigated to possess hypoglycemic activity in streptozotocin-induced diabetes in rats (Nirmala et al., 2009). Aside this, information about the anti-diabetic effects of *B. alba* is scanty. In the light of this, this work was carried out to evaluate the anti-diabetic effect of aqueous extract of *B. alba* leaves in alloxan-induced diabetic albino rats.

MATERIALS AND METHODS

Animals

A total of 30 male Wistar albino rats with weight range of 100 to 150 g purchased from the Department of Biochemistry, Bowen University, Iwo, Osun State, Nigeria were used in this study. The animals were kept in cages under standard conditions (temperature, 25±2°C, 12 h light and 12 h dark cycle) in the animal house of the Physiology Department, Faculty of Basic medical science, Bowen University, Iwo, Osun State, Nigeria. All animals were fed with commercially formulated rat feed and water *ad libitum*.

After randomization into various groups, the rats were acclimatized for a period of 2 weeks in the environment before the initiation of the experiment. Their cages were cleaned of waste daily. All procedures involving the use of animals in this study complied with the guiding principles for research involving animals as recommended by the declaration of Helsinki and the Guiding principles in the care and use of animals (World Medical Association, 2002).

Plant materials

The fresh leaves of *B. alba* (Indian spinach) were procured from Odori market in Iwo, Osun state, Nigeria. The plant materials were identified and authenticated by the Chief Herbarium Officer of the Department of Biological Sciences, Bowen University, Iwo, Osun state, Nigeria. The leaves were washed in tap water and shade-dried after which they were reduced into fine powder by grinding. 100 g of the powered leaves was stirred into 1000 ml of boiling distilled water. Boiling was allowed to continue for 5 min. The mixture was kept off the hot plate, for 30 min to allow it to infuse. It was then filtered using cheese cloth. The filtrate was then concentrated by evaporation to dryness using a water bath to obtain the solid mass. The extract was then dissolved in normal saline and used for the study.

Preparation of standard drug (metformin)

Five grams of metformin was obtained from the Pharmacy Unit of Bowen University clinic, Iwo, Osun state, Nigeria. The tablets were then grounded to fine powder. The powder was then dissolved in 25.70 ml of distilled water to get a stock solution of 19.20 g/dl.

Induction of diabetes mellitus

The animals were weighed and injected via intraperitoneal route; 100 mg/kg of alloxan dissolved in distilled water (Carvalho et al., 2003) using insulin syringes. Diabetes mellitus was confirmed after 72 h of alloxan injection by testing the fasting blood glucose levels in the blood obtained from the tail vein of the animals using glucometer and glucose test strip. The result of blood glucose measurement by glucometer correlates excellently well with the result obtained from standard laboratory methods (Ajala et al., 2003). The accuracy of the test result was confirmed by the use of glucose test kit.

Table 1. The fasting blood glucose levels in different experimental groups of rats (n=5).

Treatment mode	Group I (healthy control)	Group II (diabetic control)	Group III (DM + *B. alba* 100 mg/kg)	Group IV (DM + *B. alba* 200 mg/kg)	Group V (DM + metformin)
Baseline	76.2 ± 3.20	65.2 ± 5.48	60.2 ± 4.83	77.0 ± 3.54	63.5 ± 3.27
Week 0	75.7 ± 4.13	336.7 ± 4.79*	310.5 ± 5.36*	371.0 ± 7.28*	368.2 ± 1.03*
Week 1	60.0 ± 5.12	396.0 ± 5.29*	218.0 ± 4.65 [a]*	177.4 ± 3.76[a]*	138.4 ± 1.59[a]*
Week 2	69.5 ± 4.58	392.0 ± 5.22*	228.0 ± 4.40 [a]*	134.8 ± 2.48[a]*	109.6 ± 9.42[a]
Week 3	80.2 ± 4.41	380.2 ± 5.05*	232.8 ± 5.22 [a]*	127.9 ± 5.78[a]	100.2 ± 5.51[a]

Experimental design

Thirty (30) wistar rats were grouped into five (5) different groups containing six rats per group. Each group was kept in different cages. The grouping was done as follows: group I, healthy control (HC): they were neither induced with diabetes nor given any treatment throughout the experiment; group ii, diabetic control (DC): they were induced with diabetes but not given any form of treatment throughout the experiment; group III (DM+low dose of *B. alba*): the rats were induced with diabetes and treated with *B. alba* aqueous leaf extract at a low dose of 100 mg/kg; group IV (DM+high dose of *Basella alba*): the rats were induced with diabetes and treated with *B. alba* leaf extract at a high dose of 200 mg/kg; Group V (DM+metformin): the rats were induced with diabetes and treated with a standard drug (metformin) at a dose of 100 mg/kg.

Administration of drugs

Both metformin and *B. alba* were administered via the oral route with the aid of an oropharyngeal canular. The rats were handled appropriately to restrict movement and prevent trauma to the rats during drug administration. The drugs were administered for a period of three weeks.

Measurement of fasting blood glucose level

Baseline fasting blood sugar was recorded after the two weeks of acclimatization in all rats and after diabetes mellitus has been induced in the test groups. The fasting blood glucose (FBG) levels were measured using glucometer by obtaining blood samples from the tail vein and recorded weekly in all the groups.

Statistical analysis

The results were tabulated as mean ± Standard Error of Mean (SEM). The one way ANOVA was used to analyze the data, followed by a post-hoc test (LSD). The results are considered significant at $p<0.05$.

RESULTS

Effect of *basella alba* leaf extract and metformin on fasting blood glucose in alloxan-induced diabetic rats

The results of this experiment are shown in Table 1. Diabetes mellitus was induced at the beginning of week 0, which represents the values after induction. Rats with FBG level above 200 mg/dl were considered as having diabetes mellitus (Carvalho et al., 2003). The results show that normal rat chow has no effect on fasting blood glucose in non-diabetic healthy (Group I) and diabetic (Group II) control rats. There was sustained hyperglycaemia in diabetic control (group II) throughout the experiment.

The mean FBG levels in group II, III, IV and V were significantly higher than that of group I (healthy control group) ($p<0.05$) during week 0 and week 1. There were also increases in mean FBG levels in groups II, III, and IV (392.0.0±5.22 mg/dl, 228.0±4.40 mg/dl and 134.8±2.48 mg/dl) compared to healthy control group (59.5±4.58 mg/dl) in week 2 and this is statistically significant ($p<0.05$).

Group V showed no statistical difference when compared to the healthy control group in week 2. FBG in groups II and III (380.2±5.05 and 232.8±5.22 mg/dl) were significantly higher ($p<0.05$) than the healthy control group (80.2±4.41 mg/dl) while there was no statistical increase in group IV and V in week 3.

As depicted in Table 1, FBG levels in Group III, IV and V were significantly decreased ($p<0.05$) when compared to group II (diabetic control). The decreases lasted throughout the treatment period. There were no statistical differences between Group IV and V while there were statistical differences ($p<0.05$) between group III and V from weeks 1 to 3.

DISCUSSION

The anti-diabetic effects of aqueous leaf extract of *B. alba* and Metformin on alloxan-induced diabetes mellitus was examined in this study. The healthy control group has normal fasting blood glucose level. Therefore, this showed that normal rat chow has no effect on the fasting blood glucose levels. The increases in FBG levels above 200 mg/dl in groups II, III, IV and V, which were significant when compared to healthy control group confirmed induction of diabetes mellitus (Carvalho et al., 2003) in the test rats. Diabetic rats treated with *B. alba* aqueous leaf extract significantly had lower FBG levels when compared to the diabetic control group. The anti-hyperglycaemic effect of *B. alba* was noticed at two

different levels, for two different doses. It was observed that *B. alba* leaf extract had a more pronounced effect at a high dose than when administered at a low dose. The decrease in FBG levels in diabetic rats treated with *B. alba* may be due to the regeneration of beta cells of the pancreas by the presence of anti-oxidants (Duke and Ayensu, 1985; Nirmala et al., 2009; Olajire and Azeez, 2011) in the plant which are known to scavenge the free radicals produced by oxidative damage in the disease state (Olmendilla et al., 1997; Bamidele et al., 2010; Nirmala et al., 2011). Other reasons which may account for the reduction in the FBG levels observed may possibly include: inhibition of glucose absorption, increase sensitivity of receptors to insulin and stimulation of peripheral glucose uptake. Although, the present findings suggest the presence of anti-diabetic compound(s) in the leaf extract of *B. alba*, the exact mechanism of this effect is still speculative and requires further studies for clear understanding. The reduction in the FBG levels observed in the present work had been also reported by Nirmala et al. (2009) in another species of the plant (*B. rubra*).

The result of this study shows that three weeks of treatment is not enough time for treating diabetes mellitus if *B. alba* aqueous leaf extract is administered at a low dose of 100 mg/kg but if the dose is increased as high as 200 mg/kg, three weeks may be enough to treat diabetic rats and return the FBG levels near normal level.

Metformin however, had the greatest reduction effect on FBG level. Evident drop in FBG levels were noticed in the diabetic rats treated with Metformin from the first week to the third week of treatment. The FBG levels in Metformin-treated diabetic rats nearly appeared the same with the values obtained in the healthy control rats. The reduction in FBG levels in metformin-treated diabetic rats observed was similar to the earlier reports (Stalin et al., 2012; Shareef et al., 2013). Metformin at 100 mg/kg per body weight slightly reduced FBG levels than *B. alba* at 200 mg/kg per body weight in diabetic rats. This suggested that at high dose (200 mg/kg) used in this study, *B. alba* appears to have effect similar to metformin.

In conclusion, based on the results of this current work, aqueous leaf extract of *B. alba* has anti-diabetic effect in alloxan-induced diabetic rats. At a dose of 200 mg/kg, the anti-diabetic effect of aqueous leaf extract of *B. alba* is comparable to that of metformin-treated diabetic rats. Thus, *B. alba* leaf consumption as a vegetable should be encouraged to manage or treat diabetes mellitus.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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